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Supporting Information

A modular approach for multifunctional polymersomes with controlled adhesive properties

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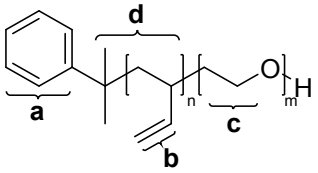
Materials and Instrumentation

All materials and solvents, unless stated otherwise, were purchased from Sigma Aldrich. Butadiene and ethylene oxide were supplied by GHC Gerling. Borosilicate glass capillaries were supplied by Hilgenberg. Dialysis tubes were obtained from Spectrumlabs. The LiposoFast Basic setup as well as the polycarbonate membranes were purchased from Avestin. Chromeo 546 azide was purchased from SantaCruz Biotechnology. 4,4-Difluoro-5,7-dimethyl-4-bora-3A, 4A-diaza-s-indacene-3-propionyl ethylenediamine hydrochloride (BODIPY Amine dye) was supplied by ThermoFisher Scientific. Fluorescence Correlation Spectroscopy (FCS) measurements were carried out on a commercial setup (Zeiss, Germany) consisting of the module ConfoCor 2 and an inverted microscope model Axiovert 200 with a Zeiss C-

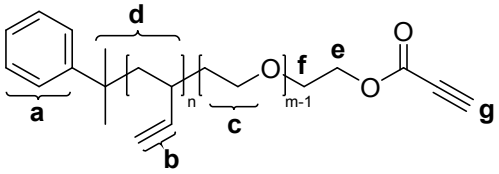
Apochromat 40×/1.2W water immersion objective Zeiss Mikroskop (Axiovert 200, ConfoCor2). BODIPY amine was excited using an Ar^+ laser (λ_0 = 488 nm) and its emission was detected in the range λ_{em} = 505 - 550 nm. Chromeo azide was excited using a He-Ne laser (λ_0 = 543 nm) and the emission detected in the range λ_{em} = 560 - 615 nm. The size of the observation volumes was calibrated using reference dyes with known diffusion coefficients, Alexa 488 and Rh6G respectively. Confocal Laser Scanning Microscopy (CLSM) measurements were performed on a TCS SP5 (Leica) using a 458 nm Argon laser, a 561 nm DPSS laser and a HCX PL APO CS 63x water objective. ^{1}H NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer. All spectra were recorded at room temperature in $CDCl_3$. ^{1}H spectra were processed with MestReNova 10.1. ^{1}H DOSY spectra were processed with TopSpin 3.0. Size exclusion chromatography (SEC) measurements were carried out in THF. The sample concentration was 1 g L^{-1}. Three SDV columns (PSS) with a particle size of 10 µm and pore sizes of 106, 104 and 500 Å and a 1260 RID Shodex RI-101 detector (ERC) were employed. Calibration was achieved using PI standards provided by Polymer Standards Service. The eluent was used at 30 °C and a flow rate of 1 mL min^{-1}. Light scattering measurements were performed on an ALV spectrometer consisting of a goniometer and an ALV-5004 multiple-tau full-digital correlator (320 channels) which allows measurements over an angular range from 30° to 150. A He-Ne Laser (λ_0 = 632.8 nm) is used as light source. For temperature controlled measurements the light scattering instrument is equipped with a thermostat from Julabo. Diluted dispersions were filtered through low protein binding hydrophilic PTFE membrane filters with a pore size of 0.45 µm (LCR Millipore). Measurements were performed at 20°C at 9 (dynamic) resp. 25 (static) angles ranging from 30° to 150°.

Characterization data of block copolymers:



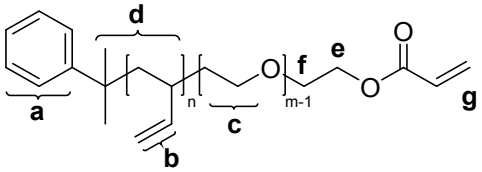
^{1}H NMR (300 MHz, CDCl3): δ 7.24-7.07 (m, superimposed by solvent, **a**), 5.58-4.84 (m, 3nH, **b**), 3.64 (s, 4mH, **c**). 2.27-0.77 (m, 3n+6H, **d**).

Alkyne Functionalisation



^{1}H NMR (300 MHz, $CDCl_3$): δ 7.24-7.07 (m, superimposed by solvent, **a**), 5.58-4.84 (m, 3nH, **b**), 4.34 (t, 2H, **e**), 3.64 (s, 4(m-1)H, **c**). 3.72 (t, 2H, **f**), 2.92 (s, 1H, **g**), 2.27-0.77 (m, 3n+6H, **d**).

Acrylate Functionalisation



^{1}H NMR (300 MHz, $CDCl_3$): δ 7.24-7.07 (m, superimposed by solvent, **a**), 6.47-5.84 (m, 3H, **g**), 5.58-4.84 (m, 3nH, **b**), 4.32 (t, 2H, **e**), 3.64 (s, 4(m-1)H, **c**). 3.74 (t, 2H, **f**), 2.27-0.77 (m, 3n+6H, **d**).

Self-assembly

Self-assembly via solvent displacement

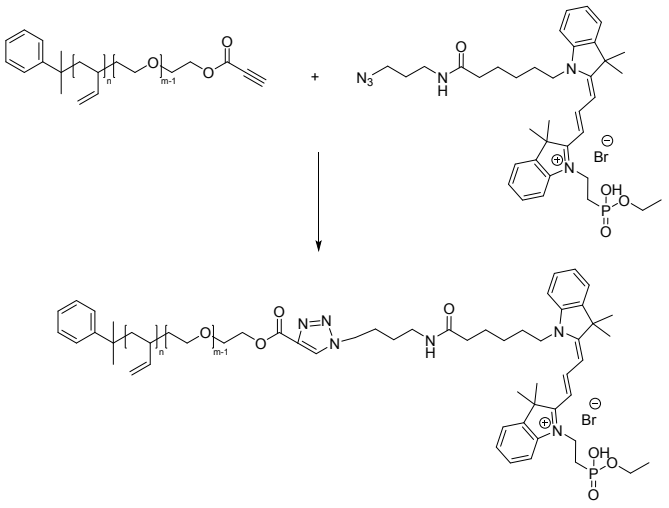
5 mg of polymer were dissolved in THF (2 mL), which is a good solvent for both blocks of the block copolymer, and placed in a dialysis tube (1000 MWCO) and dialysed against water (400 mL) overnight to induce the self-assembly process. The formerly clear THF solution turns opaque in water, indicating the self-assembly.

Extrusion

After self-assembly samples were extruded to reduce their polydispersity and/or size. MilliQ grade water was used for all steps. The LiposoFast setup was cleaned using ethanol, THF and water. Polycarbonate membranes were purged with water. Samples were extruded 11 times in volumes of up to 1 mL starting with a 1000 nm membrane, then a 400 nm membrane and finally a 200 nm membrane. Between different membranes, the setup was cleaned using water.

Modification with Chromeo 546 azide

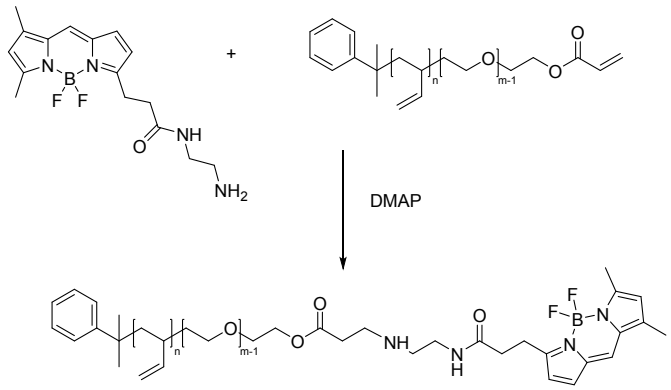
A stock solution of Chromeo 546 azide in MilliQ grade water was prepared (5.66×10^{-7} mol/mL) and diluted further if necessary. 0.5 equivalents of the dye (respective to the amount of alkynes) were added and allowed to react overnight. The functionalisation was proven by FCS measurements (cf. Figure 4).



Scheme S1: Reaction scheme for the modification of PB-*b*-PEO with Chromeo-azide.

Modification with BODIPY Amine

A stock solution of BODIPY Amine dye in MilliQ grade water was prepared (7.42E-7 mol/mL) and diluted further if necessary. 0.5 eq dye (respective to the amount of acrylates), 0.5 eq NaOH and 0.25 eq DMAP were added to the sample and allowed to react overnight. The functionalisation was proven by FCS measurements.



Scheme S2: Reaction scheme for the modification of PB-*b*-PEO with BODIPY-amine.

Orthogonal Labelling

Depending on whether samples contained an alkyne or acrylate functionality (or both), they were labelled using Chromeo 546 azide or BODIPY Amine (or both). Sample aliquots of 5 or 10 µL were used for labelling. The amount of functional groups was calculated using the following equation

$$n_{\mathrm{fg}} = \frac{M_{\mathrm{MF,\,aliquot}}}{M_{\mathrm{MF,\,total}}} * m_{\mathrm{fg,\,total}} * \ FD * \frac{1}{M_{\mathrm{n}}} \tag{9}$$

with n_{fg} the amount of functional groups, $M_{MF,\ aliquot}$ and $M_{MF,\ total}$ the mass of the middle fluid in the aliquot and the prepared solution, respectively. $m_{fg,total}$ is the amount of functional polymer. *FD* is the degree of functionalisation and M_n is the molecular weight of the polymer. $M_{MF,\ aliquot}$ is calculated from the flow rates and the run time *FD* and M_n are calculated from NMR.

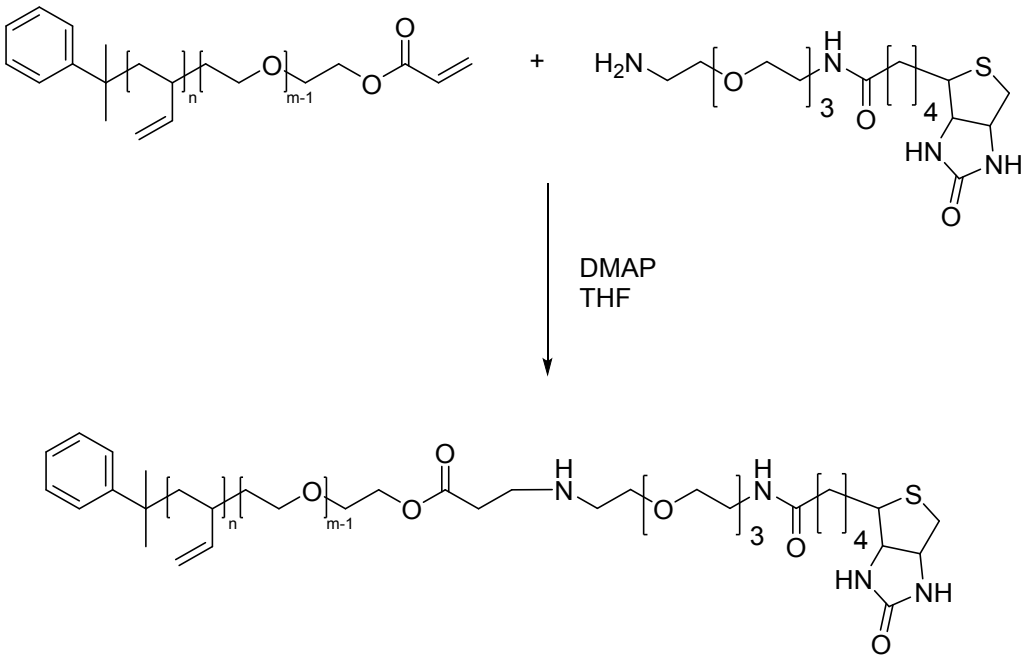
Dye labelling

Sample preparation and imaging

Vesicles from PB_{237}-*b*-PEO_{101} and PB_{237}-*b*-PEO_{101}-alkyne were reacted with chromeo azide. To this end, 20 µL vesicle sample (1.46×10^{-11} mol alkyne) were mixed with 2.46 µL chromeo azide stock solution (2.95×10^{-9} mol/mL. 0.5 eq) and left to react overnight. Vesicles from PB_{237}-*b*-PEO_{101} and PB_{237}-*b*-PEO_{101}-acrylate were stained with BODIPY amine. The vesicle sample (20 µL, 6.98×10^{-11} mol acrylate) were mixed with BODIPY amine (4.71 µL, 7.42×10^{-9} mol/mL, 0.5 eq), NaOH (3.49 µL, 0.1×10^{-4} M, 0.5 eq) and DMAP (2.44 µL, 8.19×10^{-9} mol/mL, 0.25 eq). The reaction took place overnight. To minimise bleaching of the dyes, samples were covered in aluminum foil at all times. Samples were first measured separately, then they were mixed and aliquots were measured at certain points in time. For imaging, 2 to 5 µL sample was placed in an ibidi µ-Slide. Images with at least three relevant regions of interest were measured at different points in the sample. A spectrum was recorded over the same area. Regions of interest were selected over the relevant objects and the water phase for background information. Some samples drifted while recording the spectrum, in this case the ROIs were shifted accordingly for the emission maximum of each dye.
For data correction, a vesicle sample made from PB_{237}-*b*-PEO_{101} and PB_{237}-*b*-PEO_{101}-alkyne (5 µL) was mixed with different amounts of BODIPY amine (0.25 eq, 0.5 eq, 1 eq and 2 eq) and measured the same way.

Biotin Functionalisation

PB-*b*-PEO-acrylate (11.1 mg, 1.46 x 10^{-6} mol acrylate) was dissolved in THF (4 mL) and dialysed against MilliQ grade water (600 mL) overnight using a 1000 MWCO dialysis tube. The resulting vesicle dispersion was mixed with amine-functionalised biotin (15.28 mg, 3.65 x 10^{-5} mol, 25 eq) and DMAP (4.46 mg, 3.65 x 10^{-5} mol, 25 eq) and stirred at 800 rpm for 5 days. Unreacted biotin and DMAP was removed by dialysis against MilliQ grade water (2 L, 2x changed over 5 days) using a 1000 MWCO dialysis tube. The purified sample was obtained after freeze-drying (8.0 mg, cf. Figure S1).



Scheme S3: Reaction scheme for the modification of PB-*b*-PEO-acrylate with Biotin-amine.

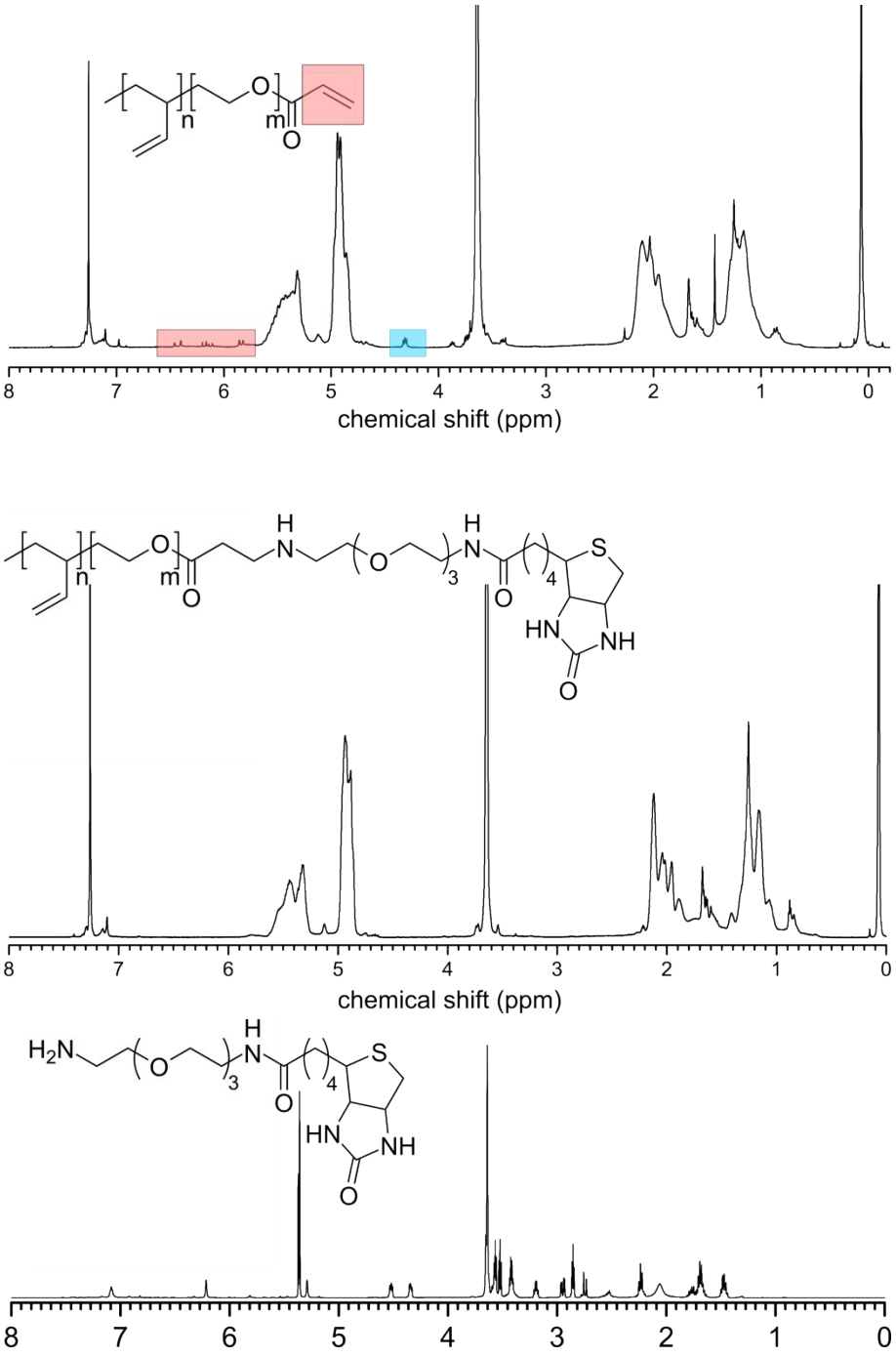


Figure S1. ^{1}H NMR (500 MHz, 298 K) spectra of PB-*b*-PEO-acrylate (top) and biotin-functionalised PB-*b*-PEO (middle) and amine-functionalised biotin (bottom) in $CDCl_3$.